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(54) Title: METHOD AND PHARMACEUTICAL COMPOSITION FOR REGULATING LIPID CONCENTRATION

(57) Abstract

The present invention is a combination of an ACAT inhibitor, for example, sulfamic acid, [[2,4,6,-tris(1-methylethyl)phenyl]acetyl]-, 2,6-bis(1-methylethyl)phenyl ester, and an HMG-CoA-reductase inhibitor, for example, atorvastatin, effective for lipid regulation. The combination of agents results in a greater reduction in plasma VLDL and LDL cholesterol and increases HDL cholesterol than either alone resulting in a less atherogenic lipoprotein profile. The combination is useful in the treatment of patients with or at risk of developing ischemic syndromes in order to restore endogenous vascular endothelium-dependent activities.

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METHOD AND PHARMACEUTICAL COMPOSITION FOR REGULATING LIPID CONCENTRATION

BACKGROUND OF THE INVENTION

The treatment of patients with or at risk of developing ischemic syndromes with doses of an HMG-CoA reductase inhibitor to lower total and LDL cholesterol is known. This is done in order to restore endogenous vascular endothelium-dependent activities including, but not limited to vasodilatory responses modulating vascular tone and blood flow, antiadherent properties of the blood vessel wall, and anticoagulation of platelets (International Publication Number WO 95/13063).

There is evidence from animal models that compounds which inhibit the enzyme, acyl-coenzyme A:cholesterol acyltransferase (ACAT) will be effective anti-atherosclerotic agents, (Curr. Med. Chem., 1994;1:204-225). It is well-established that when the majority of cholesterol in plasma is carried on apolipoprotein B-containing lipoproteins, such as low-density lipoproteins (LDL-C) and very-low-density lipoproteins (VLDL-C), the risk of coronary artery disease in man is increased (Circulation, 1990;81:1721-1733). Conversely, high levels of cholesterol carried in high-density lipoproteins (HDL-C) is protective against coronary artery disease (Am. J. Med., 1977;62:707-714). Thus, a drug which reduces the levels of atherogenic LDL-C and VLDL-C and elevates levels of protective HDL-C will produce a less atherogenic lipoprotein profile and thus a beneficial effect on atherosclerotic disease and its complications. This beneficial effect was demonstrated in man in the Helsinki Heart Study with the lipid regulator gemfibrozil which decreased LDL-C, increased

HDL-C, and reduced the incidence of coronary artery disease (N. Engl. J. Med., 1987;317:1237-1245).

SUMMARY OF THE INVENTION

We have now shown that a combination of ACAT inhibitor and HMG-CoA reductase inhibitor when administered in a chow/fat diet results in a greater reduction in apo B-containing liproprotein than either alone and that a normalization of the plasma lipoprotein profile can be achieved. This means the combination treatment results in plasma lipoprotein profile associated with a decreased risk of coronary artery disease.

We have also shown that a combination of ACAT inhibitors and HMG-CoA reductase inhibitors reduces the cholesteryl esters (CE) enrichment of pre-existing atherosclerotic lesions to the same extent as the HMG-CoA reductase inhibitor alone but that the histologic character of the atherosclerotic lesions is less complicated. This means that the lesions are less prone to induce myocardial infarction.

DETAILED DESCRIPTION OF THE INVENTION

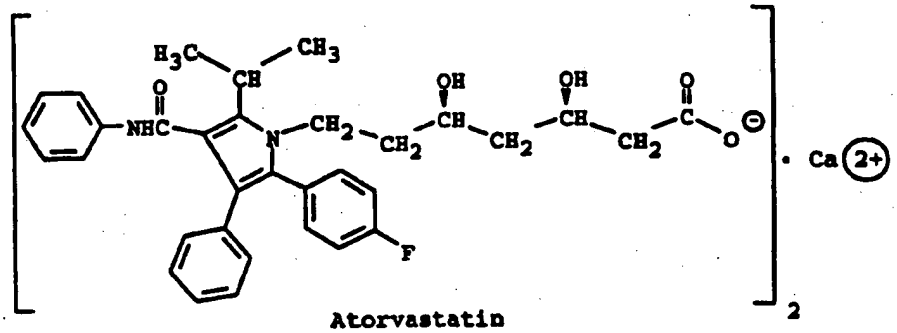
The novel method of treatment of this invention and the novel pharmaceutical compositions comprise the administration to a patient at risk of developing atherosclerosis or a patient in whom the disease has been diagnosed with an ACAT inhibitor and HMG-CoA reductase inhibitor which will restore endogenous vascular endothelium-dependent activities including improving the normal dilation capacity of the endothelium. This method may be used to induce

vasodilation to modulate vascular tone and blood flow. Other improvements in vascular endothelium-dependent activities include decreasing the adherent properties of the blood vessel walls and decreasing the coagulation of platelets. Suitable subjects for the method of the present invention include those individuals who currently exhibit symptoms of atherosclerosis and those who are at risk of developing various acute ischemic syndromes including individuals with high blood pressure, diabetes, or hyperlipidemia, and individuals who smoke.

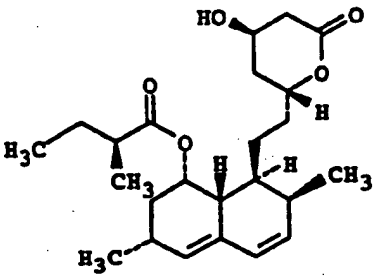
The various acute ischemic syndromes that may be treated by the method of the present invention include: angina pectoris, coronary artery disease (CAD), hypertension, cerebrovascular accidents, transient ischemic attacks, chronic obstructive pulmonary disease, chronic hypoxic lung disease, pulmonary hypertension, renal hypertension, chronic renal disease, microvascular complications of diabetes, and vaso-occlusive complications of sickle cell anemia.

An HMG-CoA reductase inhibitor for use in the novel method may be selected from atorvastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and rivastatin; preferably atorvastatin, lovastatin, or simvastatin; most preferably atorvastatin.

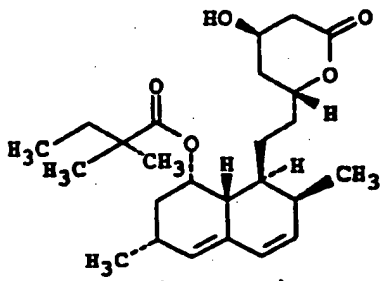
HMG-CoA reductase inhibitors are known to function as antihypercholesterolemic agents. They reduce hepatic cholesterol biosynthesis by inhibiting the enzyme HMG-CoA reductase which catalyzes the early, rate-limiting step in the biosynthesis of cholesterol, the conversion of hydroxymethylglutarate to mevalonate. Known HMG-CoA reductase inhibitors include atorvastatin MEVACOR® (lovastatin), ZOCOR® (simvastatin), PRAVACHOL® (pravastatin), LESCOL® (fluvastatin), and rivastatin.



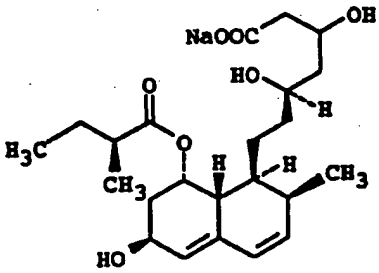
Atorvastatin



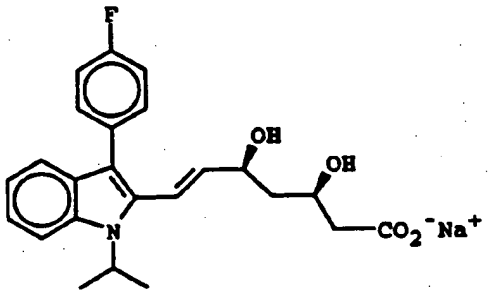
Lovastatin



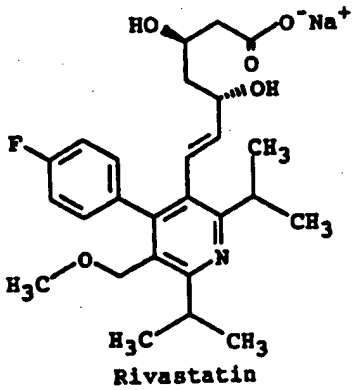
Simvastatin



Pravastatin



Fluvastatin



Rivastatin

The doses of HMG-CoA reductase inhibitor contemplated for use in this invention are about 5 to 80 mg per day, preferably given in single or divided doses.

Preferably, the patient is placed on a prudent lipid-lowering diet during the treatment with the HMG-CoA reductase inhibitors.

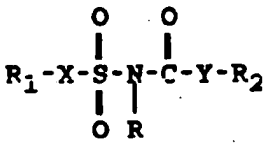
Lipid lowering therapy with HMG-CoA reductase inhibitors normalizes vascular function in patients with hypercholesterolemia and/or coronary artery disease without the requirement for significant regression of the atherosclerotic lesions. The coronary microcirculation, which demonstrates significantly impaired endothelium dependent dilatory responses in the presence of hypercholesterolemia and atherosclerotic disease, but is usually free of atheroma, is likely to show marked improvement demonstrating the ability of lipid lowering therapy to halt the progression and/or promote regression of atherosclerosis in epicardial arteries in humans.

Atorvastatin is disclosed in United States Patent Number 5,273,995. Related compounds are disclosed in United States Patent Number 4,681,893.

Lovastatin and related compounds are disclosed in United States Patent Number 4,231,938; simvastatin and related compounds are disclosed in United States Patent Number 4,450,171 and United States Patent Number 4,346,227; pravastatin and related compounds are disclosed in United States Patent Number 4,346,227 and fluvastatin and related compounds are disclosed in United States Patent Number 4,739,073; rivastatin and related compounds are disclosed in United States Patents Numbers 5,177,080 and 5,006,530.

Compounds which effectively inhibit the enzyme, acyl-coenzyme A:cholesterol acyltransferase (ACAT) prevent the intestinal absorption of dietary

cholesterol into the blood stream or the reabsorption of cholesterol which has been previously released into the intestine through the body's own regulatory action. The ACAT inhibiting compounds provide treatment of hypercholesterolemia and atherosclerosis. Such compounds include, for example, a compound of Formula I



I

or a pharmaceutically acceptable salt thereof wherein:

X and Y are selected from oxygen, sulfur and $(CR'R'')_n$, wherein n is an integer of from 1 to 4 and R' and R" are each independently hydrogen, alkyl, alkoxy, halogen, hydroxy, acyloxy, cycloalkyl, phenyl optionally substituted or R' and R" together form a spirocycloalkyl or a carbonyl;
with the proviso at least one of X and Y is $(CR'R'')_n$ and with the further proviso when X and Y are both $(CR'R'')_n$ and R' and R" are hydrogen and n is one, R_1 and R_2 are aryl;

R is hydrogen, a straight or branched alkyl of from 1 to 8 carbon atoms or benzyl;

R_1 and R_2 are each independently selected from

(a) phenyl or phenoxy each of which is unsubstituted or is substituted with 1 to 5 substituents selected from

phenyl,

an alkyl group having from 1 to 6 carbon atoms and which is straight or branched,

an alkoxy group having from 1 to 6 carbon atoms and which is straight or branched;

phenoxy,

hydroxy,

fluorine,

chlorine,
bromine,
nitro,
trifluoromethyl,
-COOH,
-COOalkyl wherein alkyl has from 1 to 4 carbon atoms and is straight or branched,
$-(CH_2)_pNR_3R_4$ wherein p is zero or one, and each of R_3 and R_4 is selected from hydrogen or a straight or branched alkyl group having 1 to 4 carbon atoms;

(b) 1- or 2-naphthyl unsubstituted or substituted with from 1 to 3 substituents selected from
phenyl,
an alkyl group having from 1 to 6 carbon atoms and which is straight or branched,
an alkoxy group having from 1 to 6 carbon atoms and which is straight or branched;
hydroxy,
phenoxy,
fluorine,
chlorine,
bromine,
nitro,
trifluoromethyl,
-COOH,
-COOalkyl wherein alkyl has from 1 to 4 carbon atoms and is straight or branched,
$-(CH_2)_pNR_3R_4$ wherein p, R_3 and R_4 have the meanings defined above;

(c) arylalkyl;

(d) a straight or branched alkyl chain having from 1 to 20 carbon atoms and which is saturated or contains from 1 to 3 double bonds; or

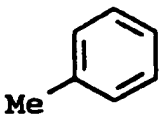
(e) adamantyl or a cycloalkyl group wherein the cycloalkyl moiety has from 3 to 6 carbon atoms;

with the provisos:

(i) where X is $(CH_2)_n$, Y is oxygen, and R_1 is a substituted phenyl, then R_2 is a substituted phenyl;

(ii) where Y is oxygen, X is $(CH_2)_n$, R_2 is phenyl or naphthyl, then R_1 is not a straight or branched alkyl chain; and

(iii) the following compounds are excluded:

X	Y	R	R_1	R_2
CH_2	O	H	$(CH_2)_2CH_3$	Ph
CH_2	O	H	CH_3	Ph
CH_2	O	H		i-Pr

The ACAT inhibitor for use in the novel method may be selected from any effective compound, especially compounds of Formula I above, especially sulfamic acid, [[2,4,6-tris(methylethyl)-phenyl]acetyl]-, 2,6-bis[(1-methylethyl)phenyl ester; 2,6-bis(1-methylethyl)phenyl-[[2,6-bis(1-methylethyl)-phenyl]sulfonyl]carbamate monosodium salt; N-(2,6-di-isopropyl-phenyl)-2-phenyl-malonamic acid dodecyl ester; N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)-dodecanamide; and N-[2,6-bis(1-methylethyl)phenyl]-N'-[[1-[4-(dimethyl-amino)phenyl]cyclopentyl]methyl urea monohydrochloride.

The doses of ACAT inhibitor contemplated for use in this invention an about 50 to 1500 mg per day, preferably given in single or divided doses.

One especially useful ACAT inhibitor is 2,6-bis(1-methylethyl)phenyl [[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate disclosed in United

States Patent Application Number 08/223,932 filed April 13, 1994, which is hereby incorporated by reference.

Other ACAT inhibitors are 2,6-bis-(1-methylethyl)-phenyl[[2,6-bis(1-methylethyl)phenoxy]-sulfonyl]-carbamate monosodium salt; and similar compounds are disclosed in United States Patent Number 5,245,068; N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl ester; and similar compounds are disclosed in United States Patent Number 5,420,339; N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; and similar compounds are disclosed in United States Patent Number 5,366,987 and divisional 5,441,975; N-[2,6-bis(1-methylethyl)phenyl]-N'-[[1-[4-(dimethylamino)phenyl]cyclo-penty]methyl]urea monohydrochloride disclosed in United States Patent Number 5,015,644 and 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl) docecanamide and similar compounds disclosed in United States Patent Number 4,716,175.

The lipid modifying and antiatherosclerotic action of 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate, atorvastatin, and the combination of both compounds was assessed in a rabbit model of atherosclerosis in which the combination of hypercholesterolemia and chronic endothelial denudation of the iliac-femoral artery promotes lesion development.

The model of atherosclerosis consists of a lesion induction phase of 15 weeks followed by an 8-week drug intervention phase. A main feature of the protocol is that after 9 weeks of a 0.5% cholesterol (C), 3% peanut (PNO), 3% coconut (CNO) oil diet plasma, cholesterol levels are normalized by feeding a 0% C, 3% PNO, 3% CNO diet prior to drug administration. The animals are randomized based on their mean plasma total cholesterol levels and administered the 0% C, 3% PNO, 3% CNO diet

either alone or containing N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide at 10 mg/kg, atorvastatin at 5 mg/kg, or N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide + atorvastatin at 10 + 5 mg/kg for the next 8 weeks.

Relative to the untreated, cholesterol-fed control, plasma total cholesterol levels were unchanged by 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate but reduced 43% and 67% with atorvastatin and 2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]-sulfamate + atorvastatin, respectively. Associated with the changes in plasma total cholesterol were marked alterations in the plasma lipoprotein distribution. 2,6-Bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate reduced % VLDL-cholesterol (VLDL-C) and increased % LDL-cholesterol (LDL-C); atorvastatin had limited effect; and upon combination treatment % VLDL-C and % LDL-C were reduced, and % HDL-cholesterol was increased.

Results are summarized in Table I below.

TABLE I. Lipoprotein Distribution Expressed as Percent of Total Plasma Cholesterol

	VLDL	LDL	HDL
Progression Control	16	60	24
2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate (10 mg/kg)	5	73	22
Atorvastatin (5 mg/kg)	14	48	38
2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate + Atorvastatin (10 + 5 mg/kg)	4	35	60

Analysis of the vascular cholesteryl ester (CE) enrichment, incidence of complex atherosclerotic lesions, gross extent of thoracic aortic atherosclerosis, and size and composition of the iliac-femoral lesion have also been performed. 2,6-Bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)-phenyl]acetyl]sulfamate had no effect on the CE enrichment of the thoracic aorta and iliac-femoral artery and on the gross extent of lesion coverage in the thoracic aorta; however, the incidence of complex fibrous plaques within the iliac-femoral artery was reduced from 50% to 14%. Atorvastatin reduced the CE enrichment of both vascular regions by 27% to 41% without changing the gross extent of thoracic lesions and incidence of fibrous plaques. 2,6-Bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)-phenyl]acetyl]sulfamate + atorvastatin had no effect on the CE enrichment of the thoracic aorta and gross extent of thoracic aortic lesions; however, the iliac-femoral CE content was reduced 23% and incidence

of fibrous plaques was decreased to 17%. Comparison of the data relative to the time zero control, i.e., prior to drug administration, atorvastatin alone and in combination with 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate significantly reduced the CE enrichment of the iliac-femoral artery. Morphometric analysis of the iliac-femoral artery revealed that atorvastatin reduced the lesion size, while the combination of atorvastatin and 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)-phenyl]acetyl]sulfamate significantly decreased the monocyte-macrophage content of the lesion without changing lesion size. 2,6-Bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)-phenyl]acetyl]sulfamate alone had no effect on the iliac-femoral lesion size or composition.

Therefore, it is clear that a combination of N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide and atorvastatin administered in a chow/fat diet results in a greater reduction in plasma apo B-containing lipoprotein than either alone and that a normalization of the plasma lipoprotein distribution is achieved. Atorvastatin not only blunts the cholesteryl ester enrichment of the vasculature but also decrease the lipid enrichment of a pre-existing atherosclerotic lesion. 2,6-Bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)-phenyl]acetyl]sulfamate + atorvastatin reduces the CE enrichment of pre-existing atherosclerotic lesions to the same extent as atorvastatin alone, but the atherosclerotic lesions are less complicated with respect to their histologic character.

For preparing the pharmaceutical compositions from the compounds of this invention, inert, pharmaceutically acceptable carriers can be either solid or

liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, and cachets.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

Powders and tablets preferably contain between about 5% to about 70% by weight of the active ingredient. Suitable carriers are magnesium dicarbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier, which is thus in association with it. In a similar manner cachets or transdermal systems are also included.

Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions, suspensions, or emulsions suitable for oral administration. Aqueous solutions for oral administration can be prepared by dissolving the active compound in water and adding suitable flavorants, coloring agents, stabilizers, and thickening agents as

desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural or synthetic gums, resins, methyl cellulose, sodium carboxymethylcellulose, and other suspending agents known to the pharmaceutical formulation art.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation containing discrete quantities of the preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of these packaged forms.

The dosage forms are well within the skill of a physician who will be familiar with such factors as time of day and other pertinent considerations.

CLAIMS

1. A method of regulating lipid concentration in a mammal in need of said treatment comprising administering therapeutically effective amounts of an acyl-CoA cholesterol O-acyltransferase (ACAT) inhibitor and an HMG-CoA reductase inhibitor.

2. A pharmaceutical composition for regulating lipid concentration in a mammal comprising a therapeutically effective amount of an acyl-CoA cholesterol O-acyltransferase (ACAT) inhibitor and an HMG-CoA reductase inhibitor together with a pharmaceutically acceptable carrier.

3. A pharmaceutical composition according to Claim 2 wherein the ACAT inhibitor is one or more compounds selected from:

Sulfamic acid, [[2,4,6-tris(1-methylethyl) phenyl]acetyl]-2,6-bis[(1-methylethyl)phenyl ester;

2,6-bis(1-methyl-ethyl)phenyl[[2,6-bis(1-methylethyl)phenyl]sulfonyl]carbamate monosodium salt;

N-(2,6-diiso-propyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester;

N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide;

2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)-docecanamide; and

N-[2,6-bis(1-methylethyl)-phenyl]-N'-[[1-[4-(dimethylamino)phenyl]-cyclopentyl]methyl urea monohydrochloride;

and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin,

simvastatin, pravastatin, fluvastatin, and atorvastatin.

4. A pharmaceutical composition according to Claim 2 wherein the ACAT inhibitor is one or more compounds selected from 2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate, N-(2,6-diiso-propyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester, and N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

5. A pharmaceutical composition according to Claim 2 wherein the ACAT inhibitor is 2,6-bis(1-methyl-ethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate and the HMG-CoA reductase inhibitor is selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

6. A pharmaceutical composition for regulating lipid concentrations in a mammal comprising a therapeutically effective amount of 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate and of atorvastatin together with a pharmaceutically acceptable carrier.

7. A method of restoring endogenous vascular endothelium-dependent activities selected from preventing coagulation of platelets, decreasing the adherent properties of blood vessel walls, and

inducing vasodilation to modulate vascular tone and blood flow in a mammal in need of said treatment which comprises administering a therapeutically effective amount of one or more ACAT inhibiting compounds and one or more HMG-CoA reductase inhibitors.

8. A method according to Claim 7 wherein the ACAT inhibitor is selected from: sulfamic acid, [[2,4,6-tris(methylethyl)phenyl]acetyl]-, 2,6-bis[(1-methylethyl)phenyl ester; 2,6-bis(1-methylethyl)phenyl-[[2,6-bis(1-methylethyl)-phenyl]sulfonyl]carbamate monosodium salt; N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl ester; 2,2-dimethyl-N-(2,4,6-trimethoxy-phenyl)docecanamide; and N-[2,6-bis(1-methyl-ethyl)phenyl]-N'-[[1-[4-(dimethylamino)phenyl]-cyclopentyl]methyl urea monohydrochloride; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

9. A method according to Claim 7 wherein the ACAT inhibitors 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate, and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

10. A method according to Claim 7 wherein 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris-(1-methylethyl)phenyl]acetyl]sulfamate and the HMG-CoA reductase inhibitor is selected from simvastatin and atorvastatin.

11. A method according to Claim 7 wherein the therapeutically effective dose of ACAT inhibitor is 50 to 1500 mg per day, and the therapeutically effective dose of HMG-CoA reductase inhibitor is about 5 to about 80 mg/day.

12. A method according to Claim 7 to prevent coagulation of platelets.

13. A method according to Claim 7 to decrease the adherent properties of blood vessel walls.

14. A method according to Claim 7 to induce vasodilation to modulate vascular tone and blood flow.

15. A method of preventing and/or treating diseases associated with endothelial dysfunction selected from: angina pectoris, myocardial infarctions, coronary artery disease, hypertension, cerebrovascular accidents, transient ischemic attacks, chronic obstructive pulmonary disease, chronic hypoxic lung disease, pulmonary hypertension, renal hypertension, chronic renal disease, microvascular complications of diabetes, and vaso-occlusive complications of sickle cell anemia which comprises administering to a mammal in preventing coagulation of platelets, decreasing the adherent properties of blood vessel walls, and inducing vasodilation to modulate vascular tone and blood flow in a mammal in need of said treatment which comprises administering a therapeutically effective amount of one or more ACAT inhibiting compounds and one or more HMG-CoA reductase inhibitors.

16. A method according to Claim 15 which comprises sulfamic acid, [[2,4,6-tris(methylethyl)phenyl] acetyl]-, 2,6-bis[(1-methylethyl)phenyl ester; 2,6-bis(1-methyl-ethyl)phenyl[[2,6-bis(1-methylethyl)phenyl]sulfonyl]carbamate monosodium salt; N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl-ester; 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[6]-thiopheno-2-carboxamide monosodium salt; 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)-docecanamide; and N-[2,6-bis(1-methylethyl)-phenyl]-N'-[[1-[4-(dimethylamino)phenyl]-cyclopentyl]methyl urea monohydrochloride; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

17. A method according to Claim 15 which comprises 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate, N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester, and N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

18. A method of stabilizing atherosclerotic lesion and preventing plaque rupture in a mammal with atherosclerosis comprising administering therapeutically effective amounts of acyl-CoA cholesterol O-acyltransferase (ACAT) inhibitor and an HMG-CoA reductase inhibitor.

19. A method according to Claim 18 which comprises administering an ACAT sulfamic acid, [[2,4,6-tris(methylethyl)phenyl]acetyl]-2,6-bis[(1-methylethyl)phenyl ester; 2,6-bis(1-methylethyl)phenyl[[2,6-bis(1-methylethyl)phenyl]-sulfonyl]carbamate monosodium salt; N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl-ester; 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[6]thiopheno-2-carboxamide monosodium salt; 2,2-dimethyl-N-(2,4,6-trimethoxy phenyl)-docecanamide; and N-[2,6-bis (1-methylethyl)-phenyl]-N'-[[1-[4-(dimethyl amino)phenyl]-cyclopentyl]methyl urea monohydrochloride; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

20. A method according to Claim 18 wherein the ACAT inhibitor is one or more compounds selected from 2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris(1-methylethyl)phenyl]-acetyl]sulfamate, N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester, and N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

21. A method of preventing ischemic sudden death in a patient at risk of the same which comprises administering therapeutically effective amounts of acyl-CoA cholesterol O-acyltransferase (ACAT) inhibitor and an HMG-CoA reductase inhibitor.

22. A method according to Claim 23 wherein the ACAT inhibitor is one or more compounds selected from sulfamic acid, [[2,4,6-tris(methylethyl)phenyl] acetyl]-, 2,6-bis[(1-methylethyl)phenyl ester; 2,6-bis(1-methyl-ethyl)phenyl[[2,6-bis(1-methylethyl)phenyl]sulfonyl]carbamate monosodium salt; N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester; 5-methoxy-3-(1-methylethoxy)-N-1H-tetrazol-5-ylbenzo[6] thiopheno-2-carboxamide monosodium salt; 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)-docecanamide; and N-[2,6-bis(1-methylethyl)-phenyl]-N'-[[1-[4-(dimethylamino)phenyl]-cyclopentyl]methyl urea monohydrochloride; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

23. A method according to Claim 23 wherein the ACAT inhibitor is one or more compounds selected from 2,6-bis(1-methylethyl)-phenyl[[2,4,6-tris-(1-methylethyl)phenyl]-acetyl]sulfamate, N-(2,6-diisopropyl-phenyl)-2-phenyl-malonamic acid dodecyl- ester, and N-(2,6-diisopropyl-phenyl)-2-(2-dodecyl-2H-tetrazol-5-yl)-2-phenyl-acetamide; and the HMG-CoA reductase inhibitor is one or more compounds selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

24. A method according to Claim 1 wherein the ACAT inhibitor is 2,6-bis(1-methylethyl)phenyl[[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate and the HMG-CoA reductase inhibitor is selected from rivastatin, lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin.

25. A method according to Claim 1 wherein the compounds 2,6-bis(1-methylethyl)phenyl[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate and atorvastatin are administered.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/15854

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/40 A61K31/41 A61K31/44 A61K31/215

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF CARDIOVASCULAR PHARMACOLOGY AND THERAPEUTICS, vol. 1, no. 2, 1996, pages 117-122, XP000618614 HEINONEN ET AL: "ATORVASTATIN, A NEW HMG-COA REDUCTASE INHIBITOR AS MONOTHERAPY AND COMBINED WITH COLESTIPOL" see abstract see page 118, left-hand column ---	1,3-11, 15-25
X	EP 0 373 507 A (SQUIBB & SONS INC) 20 June 1990	1,2,7, 11-15, 18,21
Y	see page 2, line 21-27; claims 1,2 see page 2, line 33-39; claims 4-9 see page 3, line 2-4 --- -/--	1-10, 12-25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 1997	21.03.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Kanbier, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/15854

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP 0 475 148 A (SQUIBB & SONS INC) 18 March 1992 see page 3, line 20-23; claims 2-10 see page 3, line 1-5; example 4 see page 3, line 47-50 see page 2, line 18-55	1,2,7, 11,18 1-10, 12-25
X	THE JAPANESE JOURNAL OF PHARMACOLOGY, vol. 67, no. 3, 1995, pages 195-203, XP000618560 KUSUNOKI ET AL: "STUDIES ON ACAT INHIBITORY EFFECTS AN ENZYME SELECTIVITY OF F-1394, A PANTOTHEIC ACID DERIVATIVE" see page 199, right-hand column, line 1-3, paragraph 3	1,15,18
Y	WO 94 26702 A (WARNER LAMBERT CO) 24 November 1994 cited in the application see page 7, line 22; example 5 see page 10, line 23-24; claims 1-13,16,17	1-10, 12-25
A	WO 94 09774 A (MERCK & CO INC) 11 May 1994 see page 10, line 14-21; claims 6-10 see page 16, line 5-29	1-5,7-24
A	DIABETE & METABOLISME, vol. 21, no. 2, 1995, PARIS, pages 139-146, XP000618561 DAVIGNON: "PROSPECTS FOR DRUG THERAPY FOR HYPERLIPOPROTEINAEMIA" see page 140-141 see page 142, right-hand column - page 143 see page 144, right-hand column	1-25
L	ED. J.E.F. REYNOLDS: "martindale, the extra pharmacopoeia" 1993 , THE PHARMACEUTICAL PRESS , LONDON XP002027255 see page 987-989	1,2,7, 15,18,21

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/15854

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1 and 7-25 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

See continuation sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 15854

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

A compound cannot be sufficiently characterized by its pharmaceutical profile or its mechanism of action as it is done in Claims 1,2,7,11,15,18 and 21 as: "acyl-CoA cholesterol O-acyltransferase (ACAT) inhibitor" or "HMG-CoA reductase inhibitor". The search has been executed based on compounds specifically mentioned in Claims 3-6, 8-10, 16, 17, 19, 20 and 22-25 and in Table I. Furthermore, Claims 9 and 10 are grammatically not quite clear.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte ·onal Application No
PCT/US 96/15854

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0373507 A	20-06-90	AU 4591289 A	14-06-90
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		AU 5538794 A	24-05-94